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Breakpoint Within the Nucleolus Organizer Region Resulting in a Reciprocal Translocation t(4;14)(q21;p12)

Monika Grabowski,[1] Christine Fauth,[1] Antje Wirtz,[2] and Michael R. Speicher[1]*
[1]Institut für Anthropologie und Humangenetik, LMU München, Munich, Germany
[2]Abteilung für medizinische Genetik der Kinderklinik, LMU München, Munich, Germany

Reciprocal translocations involving a break in the nucleolus organizer region (NOR) are rare. A balanced translocation in a mother and her fetus with breakpoints in the NOR at 14p12 and on the long arm of a chromosome 4 at band 4q21 is described. The rearrangement was characterized by Ag-NOR staining, multiplex fluorescence in situ hybridization (M-FISH), and FISH with rDNA probes. This and other cases with breakpoints within NORs are discussed. Am. J. Med. Genet. 92:264–268, 2000.

KEY WORDS: ectopic nucleolus organizer region; ribosomal DNA; Ag-NOR technique; multiplex-FISH

INTRODUCTION

The short arms of the acrocentric chromosomes harbor regions that specifically form and maintain the nucleoli in interphase nuclei and are therefore termed nucleolus organizer regions (NORs). These regions correspond to clusters of tandemly repeated rRNA genes and are visible on metaphase chromosomes as secondary constrictions, also called satellite stalks [Goodpasture et al., 1976; Gravholt et al., 1992; for a review see Lee et al., 1997]. Specific silver staining (Ag-NOR staining) of the NORs demonstrates their transcriptional activity [Miller et al., 1976a; Miller et al., 1976b]. Most individuals have four to seven active NORs per cell [Varley, 1977].

Displacement of NORs either through translocation or insertion to positions other than short arms of the acrocentric chromosomes, resulting in so-called ectopic NORs, is a rare event in human cells. Among these, NOR displacements dominate translocations of the whole NOR and an acrocentric chromosome short arm to a terminal region of another chromosome. These events result in satellited nonacrocentric chromosomes [for a review see Arn et al., 1995]. Less common are interstitial insertions of NORs into nonacrocentric chromosomes [Guttenbach et al., 1998, 1999; Prieto et al., 1989; Watt et al., 1984]. To our knowledge, only a small number of reciprocal translocations with a breakpoint within the NOR have been reported to date [Dev et al., 1979; Hansmann et al., 1977; Savary et al., 1991; Tomkins, 1981; Varley et al., 1981].

We present a balanced translocation originating from breaks in the NOR of a chromosome 14 and the long arm of a chromosome 4, respectively, identified in a mother and her fetus. Apart from traditional techniques, such as GTG-banding and Ag-NOR staining, chromosomes were analyzed by multiplex fluorescence in situ hybridization (M-FISH) [Speicher et al., 1996] and FISH with rDNA probes.

CLINICAL REPORT

Amniocenteses was performed at 15 weeks gestation in a 43-year-old healthy woman who previously had a healthy boy and four miscarriages. A cytogenetic analysis had been done that identified the mother as a carrier of a balanced translocation (see below). Metaphase spreads from cultures of amniocytes of her fetus were analyzed in the current pregnancy. The couple was nonconsanguineous, and there was no family history of malformations or mental retardation. A healthy child was born after an uneventful pregnancy.

Laboratory Methods

Cytogenetic analysis and Ag-NOR staining were done according to standard procedures [Howell and Black, 1980].

M-FISH was done as described previously [Eils et al., 1998]. In brief, five fluorochromes, i.e., fluorescein (FITC) and the cyanine dyes Cy3, Cy3.5, Cy5, and

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*Correspondence to: Dr. Michael R. Speicher, Institut für Anthropologie und Humangenetik, LMU München, Goethestr. 31, D-80336 München, Germany.
E-mail: speicher@fish.med.uni-muenchen.de

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Cy5.5 were used. The complex M-FISH probe-mixture was generated by pooling the painting probes according to our combinatorial labeling scheme. For example, all painting probes with FITC as part of the identifier tag were combined in a "FITC-DNA-pool," and the same was done for Cy3, Cy5, biotin, and digoxigenin. Exact probe concentrations are described in Eils et al. [1998]. The five fluorochrome DNA pools were labeled by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) [Telenius et al., 1992], mixed and ethanol-precipitated overnight. The precipitate was resuspended in a hybridization mix consisting of 50% formamide, 20% dextran sulfate, and 2 × SSC. The probe-mixture and slides were denatured and hybridized for two nights at 37°C. After posthybridization washes and blocking with 3% BSA, the biotin pool was detected with avidin-Cy3.5 (1:300; Amersham Pharmacia Biotech, Freiburg, Germany) and the digoxigenin pool with one layer of rabbit antidigoxin (1:400; Sigma, Deisenhofen, Germany) and a subsequent layer consisting of antirabbit Cy5.5 (1:500; Amersham Pharmacia Biotech). FITC-, Cy3-, and Cy5-labeled probes were imaged directly with appropriate filter sets. The slide was counterstained with DAPI, and mounted in p-phenylenediamine dihydrochloride antifade solution.

Pools of human ribosomal DNA 11.9 kb and 19.8 kb EcoRI fragments [Labella and Schlessinger, 1989] were used for the delineation of rDNA. The EcoR1 inserts were cloned into pUC9 and amplified and labeled as described above by DOP-PCR. The rDNA probes were directly labeled with diethylaminocoumarin-5-dUTP (DEAC; NEN, Köln, Germany) and hybridized as described above but without the addition of Cot-1 DNA.

FISH images were captured using the motorized epifluorescence microscope Leica DMRXA-RF8 (Leica Microsystems, Wetzlar, Germany) equipped with an eight-position filter wheel and a new generation of filter sets (Chroma Technology Corp., Brattleboro, VT) as published in Eils et al. [1998]. Images were captured using a Sensys CCD camera (Photometrics, Tucson, AZ; Kodak KAF 1400 chip). Both the camera and microscope were controlled by Leica QFISH software (Leica Microsystems Imaging Solutions Ltd., Cambridge, U.K.). M-FISH image analysis was done with the Leica MCK image analysis package (Leica Microsystems Imaging Solutions Ltd.). Results can be displayed in true colors as explained in detail in Eils et al. [1998]. For the analysis of rDNA, gray images were pseudo-colored and overlaid using the Leica QFISH software (Leica Microsystems Imaging Solutions Ltd.).

RESULTS

Cytogenetic analysis showed that both the mother and her fetus were carriers of the translocation t(4;14). The distal end of q-arm of the der(4) had a satellited appearance (Fig. 1) indicating that 14p material was translocated to this chromosome. However, GTG-banding alone did not allow the identification of the exact breakpoint on the der(14) (Fig. 1), so it was not possible to conclude whether the break had occurred in the satellite stalk or in the proximal part of the 14p-arm.

Metaphase spreads from the fetus were further analyzed by M-FISH, which verified the presence of the t(4;14) (Fig. 2) and no other structural abnormalities. However, the p-arms of acrocentric chromosomes cannot be evaluated by M-FISH because they consist of repetitive elements and hybridization to these regions is suppressed by the addition of an excess of unlabelled Cot-1 DNA to the hybridization mix. Thus, additional tests were necessary for an accurate evaluation of the breakpoints.

As expected, the rDNA probe yielded signals on all normal acrocentric chromosomes (Fig. 3a). The presence of an ectopic NOR was demonstrated on the der(4), as expected from the satellited appearance of the terminal region of the q-arm of this chromosome (Fig. 3a). On the der(14) a rDNA signal was observed at the breakpoint between chromosomes 4 and 14 (Fig. 3a). This proved that the NOR was disrupted and allowed an assignment of the breakpoint on chromosome 14 to band 14p12, and refinement of the karyotype to 46,XY,t(4;14)(q21;p12).

To test whether the NORs on the der(4) and der(14) were active, metaphase spreads of the fetus were stained with Ag-NOR (Fig. 3b). The ectopic NOR on the der(4) as well as the remaining NOR at the breakpoint of the der(14) were stained (Fig. 3b). Thus, both parts of the disrupted NOR remained active.

DISCUSSION

The translocation t(4;14)(q21;p12) observed in a mother and her fetus is unusual in that the breakpoint on the acrocentric chromosome occurred within the NOR itself. To our knowledge, only a few publications report fission of a NOR. These reported cases include a t(3;15)(q2-qter;p12) [Hansmann et al., 1977]; a t(5;13)(p13;p12) [Dev et al., 1979]; a t(9;13)(p11;p12) [Varley et al., 1981]; an unstable familial translocation with a t(11;22)(p11;p12) in a mother and a daughter, and a t(11;15)(p11;p12) in another daughter [Tomkins, 1981]; and a t(13;16)(p12;q23) [Savary et al., 1991]. In all cases the NOR was active in both the original and the translocated positions.

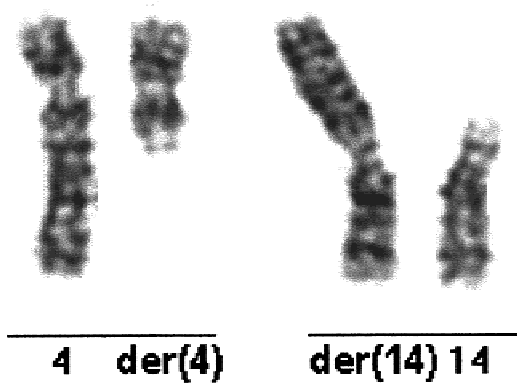

Fig. 1. GTG-banded translocation chromosomes and their normal homologues of the fetus. The satellite at the distal tip of the q-arm of the der(4) indicates that this chromosome carries material from the short arm of chromosome 14. GTG-banding alone gives no indication of the presence of the remaining NOR on the der(14).

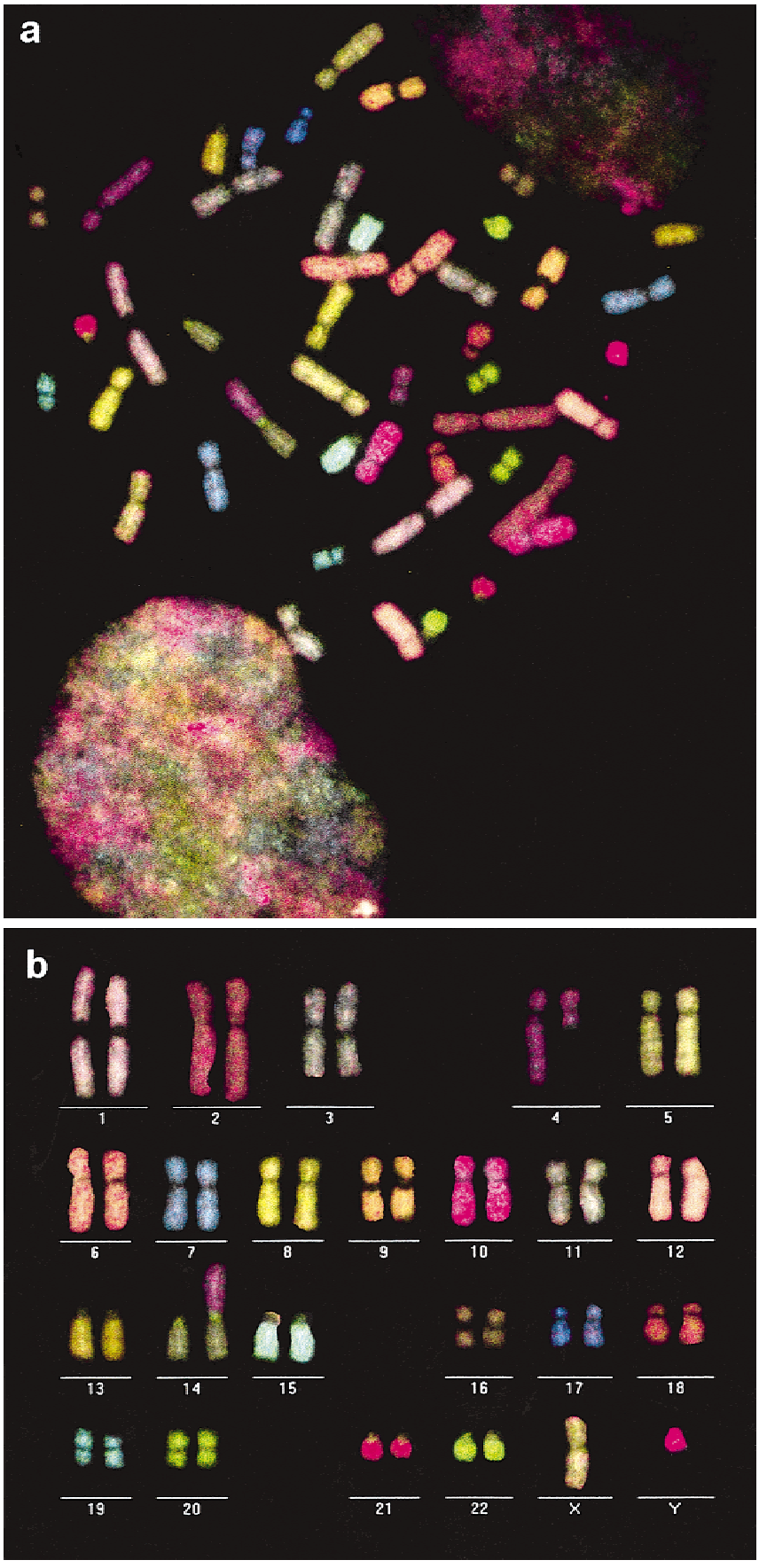

Fig. 2. Metaphase spread **(a)** and karyogram **(b)** of the fetus after hybridization of the M-FISH mix shown in true colors. The M-FISH analysis verifies the presence of the balanced t(4;14) in a male cell with no other abnormalities visible.

More frequent are translocations of NORs to a terminal region of another chromosome resulting in a satellited nonacrocentric chromosome. In this scenario, the terminal 4q is the most affected region on an autosome [Arn et al., 1995; Guttenbach et al., 1999; Miller et al., 1995]. This could indicate a special preference of the terminal 4q to recombine with the short arms of acrocentric chromosomes. The "illegitimate" breakage and reunion that produces these rearrangements may be due to the apposition of chromosomal segments containing DNA sequences with a high degree of homology [Giacalone and Francke, 1992; Lyle et al., 1995]. However, case numbers are too small to support a special 4q preference, and NORs also have been reported to be translocated to terminal regions of chromosomes 1 through 5, 9, 12, 18, X, and Y [for a review see Arn et al., 1995].

The translocation presented here is different from NOR translocations at the 4q terminal region because the break occurred in 4q21. There is no evidence for the presence of NORs interspersed at any euchromatic site within autosomes or gonosomes. Thus, translocation may occur between nonidentical sequences, euchromatin on chromosome 4, and rDNA on chromosome 14. Possible mechanisms for these rearrangements are currently not known. Whether the juxtaposition of a NOR to euchromatin may have a pathogenic effect by influencing neighboring genes is a matter of debate [reviewed in Arn et al., 1995]. Thus, the clinical implications of ectopic NORs are presently not clear and may vary depending on the location of the NOR integration. In our case neither the mother nor the child exhibited phenotypic effects, suggesting the neutral character of the chromosomal rearrangement.

Two explanations may account for the low number of reported breaks within NORs: First, the reported numbers may reflect their rarity; or second, they could be due to technical limitations resulting in difficulties in identifying them correctly. In our case, neither GTG-banding nor M-FISH contributed to the correct assignment of the breakpoint on the short arm of chromosome 14. M-FISH is a powerful tool for the identification of subtle rearrangements in pre- and postnatal diagnostics [Uhrig et al., 1999], but due to the suppression conditions, this method is incapable of classifying repetitive sequences correctly. Only FISH with rDNA-probes and Ag-NOR staining allow the correct localiza-

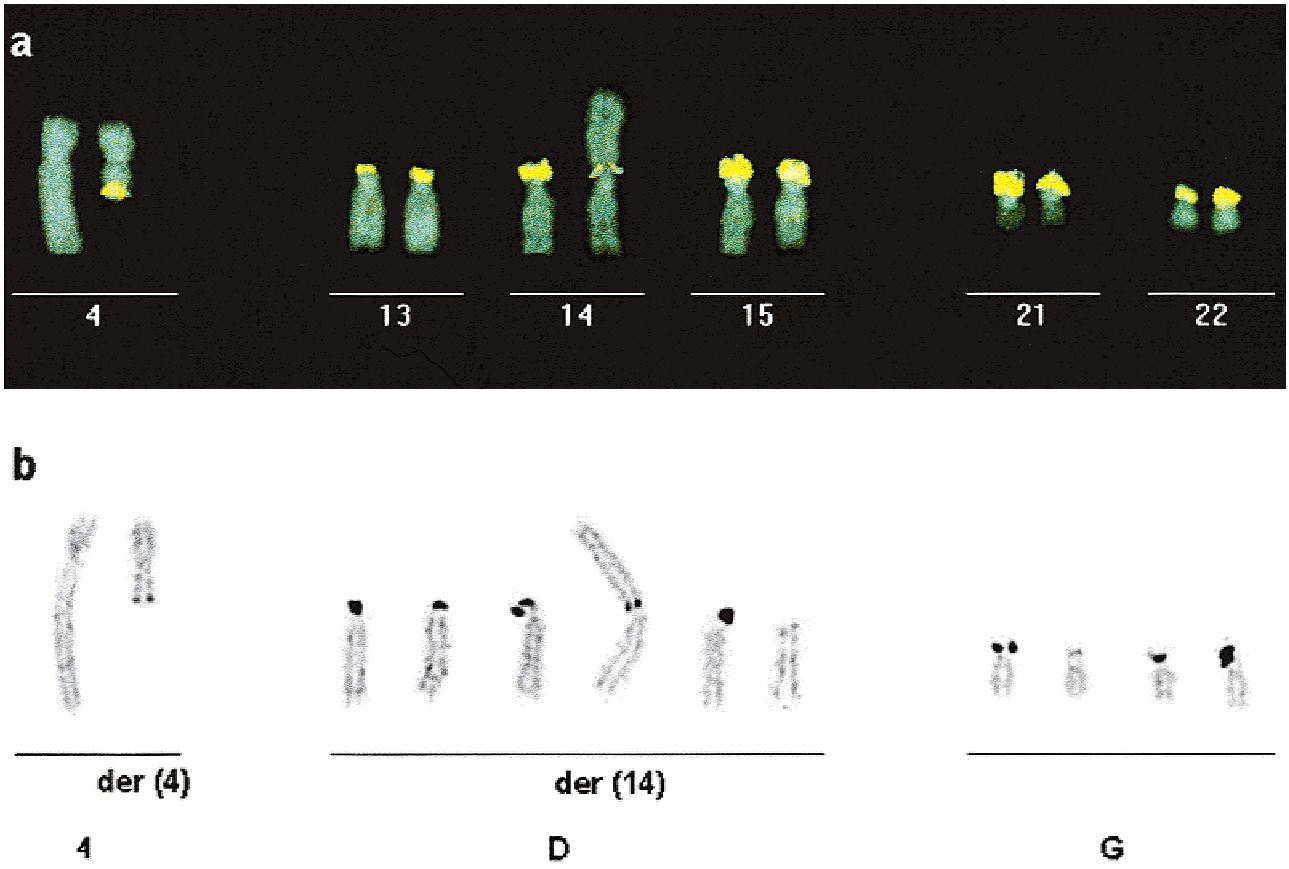

Fig. 3. Results of the rDNA hybridization **(a)** and Ag-NOR staining **(b)** on metaphase spreads of the fetus. a: The acrocentric chromosomes 13, 15, 21, and 22 show rDNA signals on the p-arms. As expected, no rDNA signal can be observed on the normal chromosome 4. The presence of an ectopic NOR at the distal tip of the der(4) is demonstrated by the rDNA signal. Both the normal chromosome 14 and the der(14) display rDNA signals. The rDNA signal on the der(14) was consistently smaller than on the der(4). Thus, the breakpoint on the short arm of chromosome 14 is within the proximal part of the NOR at band 14p12. b: NOR bearing normal acrocentric chromosomes of the D and G groups. Both the der(4) and the der(14) stain with Ag-NOR indicating their active status. Note that the Ag-NOR signals on both derivative chromosomes have approximately the same size.

tion of the break within the satellite stalk. In contrast to the Ag-NOR staining, FISH has the advantage that rDNA can be identified independently of the activity status. In addition, FISH may allow a more precise mapping of the breakpoint. As shown in Fig. 3a the rDNA signal was consistently smaller on the der(14) as compared with the rDNA signal on the der(4), indicating that the NOR was disrupted in the proximal part of the rDNA region. In contrast, Ag-NOR staining yielded signals of comparable intensities on both derivative chromosomes in all metaphase spreads (Fig. 3b). Moreover, after FISH with rDNA probes, chromosomes can be identified based on the DAPI banding, whereas Ag-NOR staining is done without a concomitant banding, which makes the accurate assignment of chromosome numbers difficult if not impossible. Thus, the strategy described represents an easy and rapid procedure for the localization of ectopic rDNA.

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